

CLAIMS

1) ~~A recombinant~~ Recombinant baculovirus ~~constituting comprising an~~
expression vector ~~that can be used for use in~~ the production of immunoglobulins
in an insect cell, ~~and characterized in that it comprises~~ said expression vector
comprising:

—~~an~~ a first expression cassette comprising a first sequence coding for at least
one part of an immunoglobulin H chain, ~~which~~ wherein said first sequence is
~~placed~~ under transcriptional control of a first baculovirus promoter,

—~~an~~ a second expression cassette comprising a second sequence coding for at
least one part of an immunoglobulin L chain, ~~which~~ wherein said second sequence
is ~~placed~~ under transcriptional control of a second baculovirus promoter; wherein

~~the~~ said first baculovirus promoter and ~~the~~ said second baculovirus
promoter ~~promoters~~ are two different promoters and are located at two different
loci.

2) ~~The recombinant~~ Recombinant baculovirus in accordance with Claim 1,
~~characterized in that~~ wherein one of ~~the~~ said first and second baculovirus
promoters is located at the site occupied in the wild baculovirus by the polyhedrin
promoter and ~~that~~ the other baculovirus promoter is located at the site occupied
in the wild baculovirus by the p10 promoter.

3) ~~The recombinant~~ Recombinant baculovirus in accordance with Claim 1 or
2, ~~characterized in that the two~~ said first and second baculovirus promoters are
strong promoters, wherein said strong promoters are at least as strong as a
polyhedrin promoter or a p10 promoter.

4) ~~The recombinant~~ Recombinant baculovirus in accordance with Claim 3,
~~characterized in that~~ wherein at least one of the first and second baculovirus
promoters is selected from the group ~~constituted by~~ consisting of:

- ~~the a~~ p10 promoter;
 - ~~the a~~ polyhedrin promoter; and
 - a synthetic promoter, referred to as Syn promoter and ~~constituted~~
 by comprising a double-strand stranded DNA fragment ~~the sequence of which,~~
~~shown in the attached sequence listing as SEQ ID NO.: 1 and SEQ ID NO.: 2, is~~
~~the following~~ having the following sequences:

5- ~~ATCAAATAAATAAGTATTTTAAAGAATTCGTACGTATTTTGTATATTAATTAAATACTATACTGTAAATAGATCG~~ -3 (SEQ ID NO:1)
 TAGTTTATTTATTCATAAAATTTCTTAAGCATGCATAAACATATAATTAATTTTATGATATGACATTTATCTAGCCTAG
 3- ~~-5 (SEQ ID NO:2)~~

5) ~~The recombinant~~ Recombinant baculovirus in accordance with ~~one of~~
~~Claims~~ Claim 1 to 4, characterized in that ~~wherein~~ each of said first and second
 expression cassette ~~cassettes~~ comprises: (i) a strong baculovirus promoter at least
as strong as a polyhedrin or p10 promoter and, under the control of ~~the said~~
 promoter: (ii) a sequence coding for a signal peptide; (iii) a sequence coding for
 a variable immunoglobulin domain; and (iv) a sequence coding for a constant
 domain of an immunoglobulin H or L chain.

6) ~~The recombinant~~ Recombinant baculovirus in accordance with Claim 5,
 characterized in that ~~the~~ wherein said sequence coding for a signal peptide placed
 under the control of the first promoter if said first expression cassette is different
 from ~~the said~~ sequence coding for a signal peptide placed under the control of the
 second promoter if said second expression cassette.

7) ~~The recombinant~~ Recombinant baculovirus in accordance with Claim 5 ~~or~~
 6, characterized in that, wherein at least one of the sequences coding for a signal
 peptide codes for a peptide that has an His-Val-Ser signal immediately upstream
 of the cleavage site used by the signal peptidase.

8) ~~The recombinant~~ Recombinant baculovirus in accordance with ~~one of~~
~~Claims~~ Claim 5 to 7, characterized in that ~~the sequence~~ wherein at least one of said
 coding for ~~the a~~ constant immunoglobulin domain is a sequence of human origin.

9) ~~An insect~~Insect cell infected by a recombinant baculovirus in accordance with ~~one of Claims~~Claim 1 to 8.

10) A method for preparing an immunoglobulin comprising the steps of:
~~culturing Procedure for the preparation of an immunoglobulin, characterized in that~~insect cells in accordance with Claim 9~~are cultured and that the~~extracting
 said immunoglobulin is ~~extracted~~from the culture medium.

11) An immunoglobulin~~Immunoglobulin, characterized in that it can be~~
 obtained by the ~~procedure in accordance with~~method of Claim 10.

12) A process for preparing~~Procedure for the preparation of a recombinant~~
 baculovirus in accordance with ~~one of Claims~~Claim 1 to 8, which ~~procedure is~~
 characterized in thatcomprising the steps of:

- ~~one prepares~~preparing a first transfer plasmid comprising a sequence coding for at least one part of an immunoglobulin H chain, under transcriptional control of a first strong baculovirus promoter; at least as strong as a polyhedrin promoter or p10 promoter

- ~~one prepares~~preparing a second transfer plasmid comprising ~~the a~~ sequence coding for at least one part of an immunoglobulin L chain, under transcriptional control of a second strong beculovirus at least as strong as a polyhedrin promoter or p10 promoter of the said baculovirus;

- ~~with the~~wherein said first and second promoters ~~being~~are two different promoters;

- ~~one carries out the~~performing homologous recombination of the two plasmids with baculovirus DNA;

- allowing replication of viral DNA in transfected cells;

- ~~after replication of the viral DNA in transfected cells, one proceeds to the~~
~~selection of the~~selecting recombinant baculoviruses that have integrated the

sequence coding for at least one part of the immunoglobulin H chain and the sequence coding for at least one part of the immunoglobulin L chain.

13) ~~Procedure in accordance with~~with the process according to Claim 12, ~~characterized in that~~wherein each of said first and second transfer plasmid ~~used~~plasmids carries an insert comprising:

- an expression cassette ~~such as defined in Claim 5 and, on both sides of this~~ cassette, comprising a strong baculovirus promoter at least as strong as a polyhedrin promoter and, under the control of said promoter, a sequence coding for a signal peptide, a sequence coding for a variable immunoglobulin domain, and a sequence coding for a constant domain of an immunoglobulin H or L chain, said expression cassette flanked on each side by baculovirus sequences homologous with those of the regions flanking the portion of the viral genome which it is the intention to replace by insertion of the said cassette ~~being replaced~~ by said expression cassette.

14) ~~The process according to~~Procedure in accordance with Claim 13, ~~characterized in that the~~wherein said baculovirus sequences are homologous with ~~those sequences~~ of the regions flanking the p10 gene or homologous with those of the regions flanking the polyhedrin gene.

15) ~~The process according to~~Procedure in accordance with Claim 14, ~~characterized in that the~~wherein said baculovirus DNA ~~with which is effected the homologous recombination of the transfer plasmids is constituted by~~comprises DNA from a baculovirus ~~that has previously been modified by insertion of two~~having a Bsu36I sit on both sides each side of the sequence coding for the p10 protein ~~(these, wherein said two Bsu36I sites being are the only Bsu36I sites for the enzyme under consideration in the genome of the said modified baculovirus)~~ DNA and wherein said baculovirus DNA is digested by the enzyme Bsu36I.